

Application No. 09/622,206

Reply to Office Action

*AMENDMENTS TO THE CLAIMS*

1. (Currently Amended) A method for quantitatively detecting an antigen in an analytical sample, said analytical sample containing an amount of antigen, which comprises:

a first step i) of providing an Fab' antibody having a uniform isoelectric point, said antibody forming an immune complex with an the antigen in an the analytical sample and being modified in one molecule by adding an amino acid sequence comprising a charged amino acid residue, and by being labeled with a fluorescent dye, and by site-specifically mutating in the cDNA expressing an Fab' antibody at least one codon encoding an amide group-containing amino acid residue in the CH1 region, into a codon encoding an amide group-non-containing amino acid residue except for cysteine, which is produced by a method comprising:

i-1) a first step of providing an Fd-chain cDNA encoding a VH region and CH1 region, and an amino acid sequence which adjoins a C-terminal of the CH1 region and comprising only one cysteine residue which is not involved in binding with an L-chain in an Fab' antibody, and an L-chain cDNA encoding the L chain of the Fab' antibody;

i-2) a second step of linking the Fd-chain cDNA and the L-chain cDNA in the expressible state to obtain a cDNA expressing an Fab' antibody;

i-3) a third step of modifying the cDNA expressing an Fab' antibody to express an amino acid sequence comprising a charged amino acid residue adjacent to a C-terminal of the L-chain, and to site-specifically mutating in the cDNA expressing an Fab' antibody at least one codon encoding an amide group-containing amino acid residue in the CH1 region, into a codon encoding an amide group-non-containing amino acid residue except for cysteine to obtain a cDNA expressing a charge modified Fab' antibody;

i-4) a fourth step of transforming a host cell with the cDNA expressing a charge modified Fab' antibody and culturing the resultant transformant to obtain an Fab' antibody having a uniform isoelectric point, the Fab' antibody being modified by adding an amino acid sequence comprising a charged amino acid sequence comprising only one cysteine residue which is not involved in binding with an L-chain adjacent to the C-terminal CH1 region, and site-specifically mutating in the cDNA expressing an Fab' antibody at least one codon encoding an amide group-

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containing amino acid residue in the CH1 region, into a codon encoding an amide group-non-containing amino acid residue except for cysteine; and

i-5) a fifth step of binding a fluorescent dye to the cysteine residue which is not involved in binding with an L-chain in the Fab' antibody having a uniform isoelectric point obtained in the fourth step;

~~a second~~ step ii) of mixing the Fab' antibody having a uniform isoelectric point with the analytical sample containing the antigen to obtain a mixture comprising the immune complex;

~~a third~~ step iii) of separating the ~~mixture~~ immune complex by performing electrophoresis in a carrier;

~~a fourth~~ step iv) of irradiating an excitation light which excites the fluorescent dye to the ~~mixture~~ immune complex separated in the ~~third~~ step iii) to cause fluorescence in the immune complex; and

~~a fifth~~ step v) of detecting the fluorescence and correlating the detected fluorescence with the amount of antigen.

2. (Canceled)

3. (Currently Amended) The method according to claim 1, wherein the fluorescent dye is bound to a cysteine residue which is not involved in binding with an ~~L chain~~ L-chain and which exists in an amino acid sequence adjacent to a C-terminal of a CH1 region of the Fab' antibody having a uniform isoelectric point.

4. (Previously Presented) The method according to claim 1, wherein the electrophoresis is performed by isoelectric focusing.

5. (Previously Presented) The method according to claim 1, wherein the electrophoresis is performed by capillary electrophoresis.

6.-21. (Canceled)

22. (Currently Amended) The method according to claim 1, wherein the amino acid sequence is added to a C-terminal of an ~~L-chain~~ L-chain of the Fab' antibody having a uniform isoelectric point.

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23. (New) The method according to claim 1, wherein the correlating the detected fluorescence involves obtaining a relative and/or absolute amount of antigen by correlating fluorescence intensity with a standard curve showing relations of various amounts of standard antigen and fluorescence intensities which represent amounts of the Fab' antibody-antigen immune complex.